IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Himanshu BRAHMBHATT *et al.*

Title: **PHARMACEUTICALLY COMPATIBLE METHOD FOR PURIFYING INTACT BACTERIAL MINICELLS**

Appl. No.: 10/602,021

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Examiner: Leon B. Lankford, Jr.

Art Unit: 1651

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DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Himanshu Brahmbhatt, declare the following:

1. I am a co-inventor named in the captioned application.

2. I am a cofounder of EnGeneIC Molecular Delivery Pty Ltd and have served as a Joint-CEO and Director of the company since its inception in February 2001. I received my Ph.D. in 1987 at the University of Adelaide, South Australia. I subsequently carried out my post-doctoral research at the Dept. de Biochemie Medicale, Centre Medicale Universitaire, Geneva, Switzerland (June 1987 - June 1988) and the National Centre for Research in Biotechnology, Braunschweig, Germany (July 1988 - Aug. 1991). I was then employed as a Research Scientist (Sept. 1991 - June 30th, 1994), Senior Research Scientist (July 1, 1994 – June 30th, 1999) and principal Research Scientist (July 1, 1999 – January 15, 2001) at CSIRO McMaster Laboratory, Division of Animal Health, Sydney, Australia. I have spent much my career studying bacterial vaccines, parasite vaccines and cancer biology and have over 12

publications in that and related fields with most of my research output in various patent applications.

3. I understand that claims 9, 11-12 and 27-40 of the above-referenced case are rejected under 35 U.S.C. § 103(a) for allegedly being unpatentable over Khatchatourians et al. In particular, I understand that Khatchatourians is cited for allegedly "teach[ing] the separation of minicells from normal, contaminating bacterial cells by inducing normal cells to filamentate followed by selective elimination of the filamentous bacteria." Office Action dated May 15, 2007, pg. 3.

4. In fact, Khatchatourians taught (a) using low levels of penicillin to inhibit cell division but not longitudinal growth of *E. coli* cells and then (b) selectively eliminating filamentous bacteria by sonic oscillation of whole cells, followed by centrifugation purification. Khatchatourians, Discussion, ¶¶1-2 (Materials and Methods, "Preparation of minicells").

5. At the time of the invention, some thirty years after the Khatchatourians publication, practitioners seeking to purify minicells did not utilize Khatchatourians' purification process or a general scheme of induced filamentation and selective elimination, which the examiner attributes to Khatchatourians.

6. In proposing his method, Khatchatourians assumed that "sonic treatment disrupts whole cells" and "does not affect minicells." *Id.* at 293. This assumption proved to be false, however. In the decades following Khatchatourians' publication, practitioners learned that sonication seriously damages minicells as well as bacterial cells. In fact, sonication became the standard method of minicell disruption in the 1980's and 1990's. A publication by Henning et al. (*Proc. Nat. Acad. Sci. USA* 76: 4360-64 (1979)), which I understand is already of record, verifies this fact.

7. Practitioners in the field in 2003 were well-aware of Khatchatourians' error and, hence, dismissed the Khatchatourians methodology as illustrating how *not* to purify bacterial minicells. Indeed, up until that time, in the more than 100 topical publications since Khatchatourians, no one used the Khatchatourians methodology.

8. Furthermore, at the time of the invention, practitioners had not embraced filamentation as a viable technique to purify minicells. Instead, practitioners typically used the differential centrifugation described by Frazer et al. (*Current Topics in Microbiology and Immunology*, 69:1-84 (1975), attached as Exhibit A). The following papers, which span the thirty years following Khatchatourians, are representative of the purification methods employed: Clark-Curtiss et al., *Methods in Enzymology*, 101:347-362 (1983); Kearns et al., *Applied and Environmental Microbiology*, 64:1837-1844 (1988); and Giddens et al., *Molecular Microbiology*, 45:769-783 (2002), which are attached as Exhibits B-D, respectively.

9. Moreover, I have seen nothing in the record which would have caused an artisan to replace Khatchatourians' centrifugation purification step with "available filters," as suggested by the examiner. The use of filtration requires knowledge of the size and uniformity of the particles to be purified. Neither of these prerequisites was known prior to our invention. Thus, before our disclosure, the skilled artisan would have had no reason to exchange Khatchatourians' centrifugation purification step with "available filters" and would have had no principled basis for expecting success from such an exchange.

10. Indeed, our discovery that minicells from a diverse range of bacteria, *e.g.* Gram negative to Gram positive bacteria, possess a uniform diameter was surprising and unexpected. Since it was well known that bacterial cell diameters varied widely, conventional wisdom held that filtration would not be useful for purifying bacterial minicells. The field's failure in the thirty years following Khatchatourians to utilize filtration to purify bacterial minicells evinces this fact.

11. Furthermore, we were the first to determine that minicells have a diameter of approximately 400 nm.

12. Having discovered the uniformity of bacterial minicells and their approximate size, we developed the inventive methods and demonstrated their effectiveness in producing minicell compositions of previously unattainable levels of purity.

13. Before the invention, "high-purity" minicell preparations contained 1 vegetative cell per 10^6 to 10^7 minicells. *See, e.g.* Clark-Curtiss et al. (Exhibit B). Since even a mouse therapeutic dose uses 5×10^8 minicells, the previously available "high-purity" minicell compositions contained 50 to 500 live parent bacterial cells/dose, which would be lethal. Thus, the inventive methods satisfy a long felt need, which spans some 30 years, for a process of obtaining pharmaceutically-acceptable compositions of bacterial minicells.

14. I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

7th March 2008
Date

Himanshu Brahmbhatt